

**BRASSICA NAPUS WITH EARLY MATURITY (EARLY NAPUS) AND
RESISTANCE TO AN AHAS-INHIBITOR HERBICIDE**

**David G. Charne
Jayantilal D. Patel
Alan Grombacher**

FIELD OF THE INVENTION

This invention is in the field of canola breeding. In particular, it relates to improved varieties of canola (*Brassica napus*) having early maturity ("Early Napus"), in combination with resistance to at least one AHAS-inhibitor herbicide.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 USC 119(a) to Canadian Application No. 2,326,283 filed November 17, 2000, the disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Canola is an important agricultural crop in Canada, the United States, Europe and Australia. Weed competition and earliness of maturity are significant limiting factors in canola crop production and quality. The challenge for plant scientists has been to develop canola varieties having superior performance with respect to these limiting factors, while at the same time having satisfactory agronomic characteristics, including yield potential, lodging resistance, oil and protein content, and glucosinolate levels that are sufficiently low for registration.

Resistance to AHAS-Inhibitor Herbicides

Herbicide resistant plants are plants that are able to survive and reproduce following exposure to herbicides at rates of application that would prevent non-

herbicide resistant varieties of the same species from surviving and reproducing.

Herbicide resistance is particularly important for *Brassica*, since many weeds, such as stinkweed, shepherd's purse, flixweed, ball mustard, wormseed mustard, hare's ear mustard and common peppergrass have a close genetic relationship with

5 *Brassica*. Therefore, it is advantageous for a cultivar to have herbicide resistance not possessed by related weeds.

Some herbicides function by disrupting amino acid biosynthesis in affected species. For example, AHAS-inhibitor herbicides (also known as ALS-inhibitor herbicides) function by inhibiting the enzyme acetohydroxy acid synthase (AHAS), the
10 first enzyme in the biosynthesis of the amino acids, isoleucine, leucine and valine. In plants with resistance to an AHAS-inhibitor herbicide, inhibition of the AHAS enzyme is prevented, thus allowing the plant to continue with normal amino acid biosynthesis. Most forms of *Brassica* are highly susceptible to AHAS-inhibitor herbicides, such as imidazolinones and sulfonylureas.

15 The development of canola with resistance to imidazolinones, such as PURSUIT™ and ODYSSEY™, was a major breakthrough in weed management technology. The imidazolinones are a family of broad spectrum herbicides which may be applied for in-crop weed control. They control a larger number of problem species than herbicides used in non-herbicide resistant varieties, and offer greater
20 management flexibility, including timing of application and tank mixing. An advantage of imidazolinone ("IMI") resistant varieties over many other herbicide resistant varieties, such as ROUNDUP READY™ (glyphosate) or LIBERTY LINK™ (glufosinate) resistant varieties, is that some imidazolinone herbicides have a soil residual which controls successive weed flushes. This provides a significant
25 advantage to farmers, because it enables them to achieve longer term weed control without a second application of herbicide. Effective weed control increases yield by reducing competition from weed species. It also improves grain quality through the elimination of cruciferous weed seeds. It may also improve weed management in other crops in the rotation, due to reduced weed pressure.

30 However, a drawback of currently available IMI resistant varieties is that they lack many of the desirable traits found in elite varieties of non-herbicide resistant

canola. In particular, none of the currently available canola varieties have the desirable combination of IMI resistance and early maturity (Early Napus). It is particularly difficult to develop varieties having IMI resistance, in combination with other desirable traits, because the inheritance of the IMI resistance trait is relatively complex. Unlike the ROUNDUP READY™ trait or LIBERTY LINK™ trait, which are controlled by single transgenes that exhibit complete dominance, the IMI resistance trait is controlled by two unlinked gene pairs having partial dominance. Swanson et al., Plant Cell Reports 7:83-87 (1989) reported the development of imidazolinone herbicide tolerant *Brassica napus* mutants using microspore mutagenesis. During the process, five fertile double-haploid *Brassica napus* mutant plants were developed. One of the mutants was tolerant to between 5 and 10 times the recommended field traits of an imidazolinone herbicide. An inheritance study indicated that two semi-dominant unlinked genes combined to produce an F1 with greater tolerance than either of the parents.

Rutledge et al., Mol. Gen. Genet. 229:31-40 (1991) proposed a model for the inheritance of the five AHAS genes in *Brassica napus*. AHAS2, AHAS3 and AHAS4 appear to be associated with the 'A' (rapa) genome and AHAS1 and AHAS5 are likely associated with the 'C' (oleracea) genome. AHAS1 and AHAS3 are expressed at all growth stages (Ouellet et al., Plant J. 2:321-330 1992) and mutant forms of AHAS1 and AHAS3 appear to be the most effective tolerance genes. AHAS2 was found to be active only in ovules and seeds. AHAS4 was found to be defective due to interrupted sequences in the middle of the coding region (Rutledge et al., Mol. Gen. Genet. 229:31-40 1991) and was not expressed in tissues examined by Ouellet et al., Plant J. 2:321-330 (1992). The last gene AHAS5, may also be defective (Rutledge et al. Mol. Gen. Genet. 229:31-40, 1991). Hattori et al. Can J. Bot: 70:1957-1963, (1992) determined that the DNA sequence of the coding regions for AHAS1 and AHAS3 were 98% identical. DNA sequences of the 5' and the 3' ends were also closely related. Few similarities were observed between the sequence of the AHAS2 compared to the AHAS1 or AHAS3 genes.

There are two effective mutations for IMI resistance in commercial use - an AHAS1 mutant (believed to be located on the C genome) and an AHAS3 mutant

(believed to be located on the A genome). The AHAS3 mutant also provides resistance to other AHAS-inhibitor herbicides, such as sulfonylureas. The complexity of the inheritance of the IMI resistance trait results in multiple phenotypes during segregating generations, which presents a significant hurdle to plant breeders.

- 5 Accordingly, there is a need to develop an AHAS-inhibitor herbicide resistant canola variety with improved performance characteristics.

Early Napus

10 Early maturity is an important trait in *Brassica napus* varieties, especially in market areas with a limited frost-free period. Late summer frosts can damage the crop before it is fully mature, resulting in elevated green seed content of the grain (a grading criterion) and increased chlorophyll in the oil (a quality problem). High green seed results in losses to the producer, while elevated chlorophyll in the oil increases processing costs, and results in a loss of value for food end users. Early Napus is also important where early maturity reduces exposure to extreme heat and drought conditions during flowering and seed-filling.

15 To be classified as "Early Napus", a variety must have an average maturity which is at least four days earlier than the average maturity of the current WCC/RRC (Western Canadian Canola/Rapeseed Recommending Committee) check varieties (DEFENDER™, EXCEL™, and LEGACY™) over two years at 11 locations in the Short Season Zone of Western Canada. No known varieties of *Brassica napus* have the desirable combination of Early Napus and resistance to an AHAS-inhibitor herbicide, such as an imidazolinone. Therefore, there is a need for a *Brassica napus* variety which combines the advantageous traits of early maturity (Early Napus) and resistance to AHAS-inhibitor herbicides.

25 Accordingly, it is an object of the present invention to provide an improved variety of *Brassica napus* having early maturity (Early Napus) and resistance to at least one AHAS-inhibitor herbicide, such as an imidazolinone. These and other objects of the invention will be apparent to those skilled in the art from the following description and claims.

SUMMARY OF THE INVENTION

This invention provides a *Brassica napus* plant which is Early Napus and resistant to at least one AHAS-inhibitor herbicide, such as an imidazolinone (e.g. imazethapyr or imazamox) or a sulfonylurea [e.g. thifensulfuron methyl (REFINE TM)].

5 In one embodiment, it relates to canola variety NS3801.

This invention also relates to tissue cultures of regenerable cells from the plants described above, as well as to the use of the tissue cultures for regenerating canola plants that are Early Napus and resistant to at least one AHAS-inhibitor herbicide, such as an imidazolinone or a sulfonylurea. It also relates to the plants
10 produced therefrom.

This invention further relates to the parts of the *Brassica napus* plants described above, including their cells, pollen, ovules, roots, leaves, seeds, microspores and vegetative parts, whether mature or embryonic. It also relates to the use of these plant parts for regenerating a canola plant that is Early Napus and
15 resistant to at least one AHAS-inhibitor herbicide, such as an imidazolinone or a sulfonylurea, and to the plants regenerated therefrom.

This invention further relates to the use of the plants described above for breeding a *Brassica* line, through pedigree breeding, crossing, self-pollination, haploidy, single seed descent, modified single seed descent, and backcrossing, or
20 other suitable breeding methods, and to the plants produced therefrom. This invention also relates to a method for producing a first generation (F1) hybrid canola seed by crossing one of the plants described above with an inbred canola plant of a different variety or species, and harvesting the resultant first generation (F1) hybrid canola seed. It further relates to the plants produced from the F1 hybrid seed.

25 This invention also relates to the use of the *Brassica napus* plants described above for producing oil and/or meal, and to the vegetable oil and meal produced therefrom. Preferably, the plant is capable of producing oil with less than 2% erucic acid and meal with less than 30 μ mol of glucosinolates per gram of defatted meal.

This invention provides substantial value to both producers and users of
30 canola by providing hitherto unavailable combinations of early maturity (Early Napus) and resistance to at least one AHAS-inhibitor herbicide. This trait combination

improves weed control, while improving or stabilizing grain quality by reducing green seed count.

DETAILED DESCRIPTION OF THE INVENTION

5 In accordance with this invention, improved varieties of *Brassica napus* having early maturity (Early Napus) and resistance to at least one AHAS-inhibitor herbicide are developed by crossing a parent with resistance to an AHAS-inhibitor herbicide with one or more parents having early maturity (Early Napus), wherein the herbicide resistant parent and the Early Napus parent(s) together have the genetic basis for the
10 complement of characteristics desired in the progeny. Self-pollination or sib-mating following crossing leads to a segregation of traits among the progeny. Progeny having the desired combination of traits are selected after exposure to one or more appropriate AHAS-inhibitor herbicides and evaluation for desirable traits over successive generations.

15 Various breeding methods may be used, including haploidy, pedigree breeding, single-seed descent, modified single seed descent, recurrent selection, and backcrossing. Because of the complex inheritance of the AHAS-inhibitor herbicide resistant trait, we have found that haploidy is the most effective breeding method. Parents having the desired complement of characteristics are crossed in a simple or
20 complex cross. Crossing (or cross-pollination) refers to the transfer of pollen from one plant to a different plant. Progeny of the cross are grown and microspores (immature pollen grains) are separated and filtered, using techniques known to those skilled in the art [(e.g. Swanson, E.B. et al., Plant Cell Reports, "Efficient isolation of microspores and the production of microspore-derived embryos in *Brassica napus*",
25 6:94-97 (1987); and Swanson, E.B., Microspore Culture in *Brassica*, pp. 159-169 in: Methods in Molecular Biology, Vol. 6, Plant Cell and Tissue Culture, Humana Press (1990)]. These microspores exhibit segregation of genes. The microspores are cultured in the presence of an appropriate AHAS-inhibitor herbicide, such as imazethapyr (e.g. PURSUIT™) or imazamox (e.g. RAPTOR™) or a 50/50 mix of
30 imazethapyr and imazamox (e.g. ODYSSEY™), which kills microspores lacking the mutations responsible for resistance to the herbicide. Microspores carrying the

mutant genes responsible for resistance to the herbicide survive and produce embryos, which form haploid plants. Their chromosomes are then doubled to produce doubled haploids.

The doubled haploids are evaluated in subsequent generations for herbicide resistance, early maturity, and other desirable traits. AHAS-inhibitor herbicide resistance may be evaluated by exposing plants to one or more appropriate AHAS-inhibitor herbicides and evaluating herbicide injury. Earliness of maturity can be evaluated through visual inspection of seeds within pods (siliques) on the plants. Some other traits, such as lodging resistance and plant height may also be evaluated through visual inspection of the plants. Blackleg resistance may be evaluated by inoculating plants with blackleg spores to induce the disease, and observing resistance to infection. Other traits, such as oil percentage, protein percentage, and total glucosinolates of the seeds may be evaluated using techniques such as Near Infrared Spectroscopy.

It is also possible to analyze the genotype of the plants, using techniques such as Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLPs), and Simple Sequence Repeats (SSRs) which are also referred to as "Microsatellites".

Evaluation and manipulation (through exposure to one or more appropriate AHAS-inhibitor herbicides, and blackleg infection) typically occurs over several generations. The performance of the new lines is evaluated using objective criteria in comparison to check varieties. Lines showing the desired combination of traits are self-pollinated to produce seed. Self-pollination refers to the transfer of pollen from one flower to the same flower or another flower of the same plant. Plants that have been self-pollinated and selected for type for many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny.

Other breeding methods may also be used. For example, pedigree breeding is commonly used for the improvement of largely self-pollinating crops such as canola.

Pedigree breeding starts with the crossing of two genotypes, each of which may have one or more desirable characteristics that is lacking in the other or which complements the other. If the two original parents do not provide all of the desired characteristics, additional parents can be included in the crossing scheme.

5 These parents are crossed in a simple or complex manner to produce an F_1 . An F_2 population is produced by selfing one or several F_1 's or by intercrossing two F_1 's (i.e., sib mating). Selection of the best individuals may begin in the F_2 population, and beginning in the F_3 the best families, and the best individuals within the best families are selected. Replicated testing of families (lines) can begin in the
10 F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F_6 and F_7), the best lines or mixtures of phenotypically similar lines commonly are tested for potential release as new cultivars.

 The single seed descent (SSD) procedure may also be used to breed
15 improved varieties. The SSD procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the population of single seeds to plant the next generation. When the population has been advanced from the F_2 to the desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a
20 population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the plants originally sampled in the F_2 population will be represented by a progeny when generation advance is completed.

 In a multiple-seed procedure, canola breeders commonly harvest one or more
25 pods from each plant in a population and thresh them together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique. The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each by hand for
30 the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seeds of a population each generation of inbreeding.

Enough seeds are harvested to make up for those plants that did not germinate or produce seed.

Backcross breeding can be used to transfer a gene or genes for a simply inherited, highly heritable trait from one line or cultivar (the donor parent) into another desirable cultivar or inbred line (the recurrent parent). After the initial cross, individuals possessing the phenotype of the donor parent are selected and are repeatedly crossed (backcrossed) to the recurrent parent. When backcrossing is complete, the resulting plant is expected to have the attributes of the recurrent parent and the desirable trait transferred from the donor parent.

Improved varieties may also be developed through recurrent selection. A genetically variable population of heterozygous individuals is either identified or created by intercrossing several different parents. The best plants are selected based on individual superiority, outstanding progeny, or excellent combining ability. The selected plants are intercrossed to produce a new population in which further cycles of selection are continued.

Regeneration of Plants

This invention also relates to the parts of the plants disclosed herein, including plant cells, tissue, pollen, ovules, roots, leaves, seeds, and microspores, whether mature or embryonic.

The plants produced in accordance with the present invention may be regenerated from plant parts using known techniques. For instance, seeds from the plants of the present invention may be planted in accordance with conventional *Brassica* growing procedures. These plants will generate further seeds following self-pollination. Alternatively, doubled haploid plantlets may be extracted to immediately form homozygous plants, using known procedures.

Brassica plants may also be regenerated using tissue culture and regeneration. Tissue culture of various tissues of canola and regeneration of plants therefrom is known to those skilled in the art. For example, the propagation of a canola cultivar by tissue culture is described in the following references: Chuong et al., "A Simple Culture Method for *Brassica* Hypocotyl Protoplasts", Plant Cell Reports

4:4-6 (1985); Barsby, T.L. et al. "A Rapid and Efficient Alternative Procedure for the Regeneration of Plants from Hypocotyls Protoplasts of *Brassica napus*", Plant Cell Reports, (Spring 1996); Kartha, K. et al. "In vitro Plant Formation from Stem Explants of Rape" Physiol. Plant, 31:217-220 (1974); Narashimhulu, S. et al., "Species Specific Shoot Regeneration Response of Cotyledenary Explants of *Brassicas*", Plant Cell Reports, (Spring 1988); Swanson, E., "Microspore Culture in Brassica", Methods of Molecular Biology, Vol. 6, Chapter 17, p. 159 (1990).

Use of *Brassica* as a Breeding Line

The *Brassica napus* plants of this invention may be used to breed a novel *Brassica* line. The combination of desired traits described herein, once established, can be transferred into other *Brassica napus* plants by known plant breeding techniques including self-pollination, crossing, recurrent selection, backcross breeding, pedigree breeding, single seed descent, modified single seed descent, haploidy, and other suitable breeding methods.

The desired traits can also be transferred between *Brassica* species, such as *B. napus*, *B. rapa* (formerly known as *B. campestris*), and *B. juncea*, using the same known plant breeding techniques involving pollen transfer and selection. The transfer of traits between *Brassica* species, such as *napus* and *rapa* by known plant breeding techniques is well documented in the technical literature (see for instance, Tsunada et al., 1980, *Brassica Crops and Wild Alleles Biology and Breeding*", Japan Scientific Press, Tokyo).

As an example of the transfer of the desired traits described herein from *napus* to *rapa*, one selects a commercially available *rapa* variety such as REWARDTM, GOLDRUSHTM, and KLONDIKETM, and carries out an interspecific cross with one of the plants from the present invention. After the interspecific cross, members of the F₁ generation are self-pollinated to produce F₂ oilseed. Selection for the desired traits is then conducted on single F₂ plants which are then backcrossed with the *rapa* parent through the number of generations required to obtain a euploid (n = 10) *rapa* line exhibiting the desired combination of traits.

In order to avoid inbreeding depression (e.g. loss of vigour and fertility) that may accompany the inbreeding of *Brassica rapa*, selected, i.e. BC₁ plants that exhibit similar desired traits while under genetic control advantageously can be sib-mated. The resulting oilseed from these crosses can be designated BC₁SIB₁ oilseed.

- 5 Accordingly, the fixation of the desired alleles can be achieved in a manner analogous to self-pollination while simultaneously minimizing the fixation of other alleles that potentially exhibit a negative influence on vigor and fertility.

- 10 This invention is also directed to methods for producing an F₁ hybrid seed by crossing a first parent *Brassica napus* plant with a second parent *Brassica* plant, wherein the first parent plant is an inbred *Brassica napus* plant, such as canola variety NS3801, which is Early Napus and resistant to at least one AHAS-inhibitor herbicide. This invention is also related to the plants produced from the F₁ hybrid seed and the cells and other parts of those plants.

- 15 Alternatively, both first and second parent *Brassica* plants can come from the same varieties. Advantageously, one of the *Brassica* varieties of the present invention is used in crosses with a different *Brassica* inbred to produce first generation (F₁) canola hybrid seeds and plants with superior characteristics and increased vigour.

- 20 Preferably when generating hybrid plants, the parent should have glucosinolate levels that are sufficiently low to ensure that the seed of the F₁ hybrid has glucosinolate levels within regulatory levels. The glucosinolate level of the seed harvested from the F₁ hybrid is roughly the average of the glucosinolate levels of the male and female parents. For example, if the objective is to obtain hybrid grain (F₂) having a glucosinolate level of less than 20 µmol/g, and one parent has a
25 glucosinolate level of 15 µmol/g, the other parent must have a glucosinolate level of 25 µmol/g or less.

Vegetable Oil and Meal

- 30 The seed of the plants of this invention may be used for producing vegetable oil and meal. The seed of these varieties, the plant produced from such seed, the hybrid canola plant produced from the crossing of these varieties with other inbred

varieties, the resulting hybrid seed, and various parts of the hybrid canola plant can be utilized in the production of an edible vegetable oil or other food products in accordance with known techniques. The remaining solid meal component derived from seeds can be used as a nutritious livestock feed. Canola variety NS3801 can be used to produce oil of improved quality, due to lower chlorophyll levels in the oil. Preferably, the oil has less than 2% erucic acid and the meal has less than 30 μmol of glucosinolates per gram of defatted meal.

A preferred embodiment of this invention is set forth below. It should be understood, however, that the invention is not limited to the specific details set forth in this example.

Development of the improved IMI resistant *Brassica napus* line, NS3801.

Generation:	Parent to F1
Seed Planted:	BULLET™ and DEFENDER™, two spring canola varieties developed by Svalof-Weibulls, and marketed commercially by Proven Seed
Seed Harvested:	94SN-9514=(BULLET™/DEFENDER™)
Method:	Parents were grown and crossing was carried out in a controlled environment in the greenhouse.
Generation:	Single cross F1 to three-way cross F1
Seed Planted:	94SN-9514= (BULLET™ x DEFENDER™) and 45A71 (Breeder code NS1471, registered imidazolinone resistant spring canola variety from Pioneer Hi-Bred, commercially available from Proven Seed)
Seed Harvested:	96SN-0564=(45A71 x (BULLET™ x DEFENDER™))
Method:	Parents were grown and crossing was carried out in a controlled environment in the greenhouse. 45A71 was used as the female parent. Approximately six female plants and more than 10 male plants were sampled in making the three-way cross. IMI

resistance was contributed by 45A71, which is homozygous for the imidazolinone resistant genes.

Generation: Three way Cross F1 to doubled haploid (F-infinity)

Seed Planted: 96SN-0564=(45A71 x (BULLET™x DEFENDER™))

5 **Seed Harvested:** 97DHS-6259

Method: Twelve plants of 96SN-0564 were planted in the growth room under controlled environment as donor plants. These plants were sprayed with the herbicide, PURSUIT™ (imazethapyr), at 1x level. Immature buds were harvested from each donor plant and were crushed in a blender to produce a slurry [as described in Swanson, E.B. et al., "Efficient isolation of microspores and the production of microspore-derived embryos in *Brassica napus*" L. Plant Cell Reports 6: 94-97 (1987); and Swanson, E.B. Microspore culture in *Brassica*, pgs. 159-169 in: Methods in Molecular Biology vol. 6, Plant Cell and Tissue Culture, Humana Press (1990)]. The slurry was then filtered through two layers of Nitex filters (48 µm pores) and collected in centrifuge tubes. The suspensions were centrifuged, decanted and washed three times for a total of 4 spins. Microspores were counted using a haemocytometer and plated in NLN medium [Lichter, R., "Induction of haploid plants from isolated pollen of *Brassica napus*, Z. Pflanzenphysiol. Bd. 105: 427-434, (1982)], containing 40µg/l PURSUIT™, at a density of 60,000 microspores per ml. Ten ml of this suspension were poured into 100x25mm petri plates wrapped with parafilm, and placed in a Percival incubation chamber at 32.5°C in darkness for 15 days. During this period the microspores carrying imidazolinone-resistant genes were expected to survive and produce embryos. After 15 days, petri plates with cotyledonary embryos were put in a rotary shaker for 6 to 13 days before being transferred to a solid 0.8% agar medium with 0.1% Gibberillic acid (GA) in petri plates.

Transferred embryos were incubated in the dark at 4-8°C for 7-10 days and removed to a Percival incubation chamber in light at 20 to 25°C for 3 to 5 weeks. Selected embryos that regenerated were placed in soil in 72 cell flats or put back onto 0.8% agar with 0.1% GA for a further 3 to 5 weeks before they were transplanted into the soil. Before flowering, plants were treated with 0.33% colchicine for 1.5 to 2.5 hours. Plant roots were washed free of soil prior to incubation in the colchicine solution. After treatment they were planted in 10 cm plastic pots. Upon flowering, plants with fertile (diploid) racemes were covered with perforated, clear plastic bags to produce selfed seeds. After flowering, bags were removed and plants were dried down, seed was harvested, cleaned and cataloged with a DHS number. Lines with 100 seeds or more were prepared for nursery evaluation.

Generation: Doubled haploid evaluation

Seed Planted: 97DHS-6259 along with the check varieties 46A72 (NS1472), 45A71 (NS1471) and 46A74 (NS2211)

Seed Harvested: In order to perform quality analysis, twenty grams of open pollinated seed was harvested from 97DHS-6259. An equal amount of seed was harvested from the check rows. After completing the evaluation and finalizing selections, seed was harvested from the entire row for each selected line including 97DHS-6259.

Method: Several hundred imidazolinone resistant spring canola doubled haploid lines, including 97DHS-6259, were planted in the breeding nursery (project X823A) for evaluation purpose. Each line was planted in a three meter long row with approximately 100 seeds/row. 46A72 was planted in every 20th row (#1, 20, 40, 60 etc.) for use as a quality check. 45A71 and 46A74, commercial imidazolinone resistant varieties from Pioneer Hi-Bred, were planted as checks in rows, 10, 50, 90 and 30, 70 110

of each range. The entire nursery was sprayed with ODYSSEY™ (a 50/50 mix of imazethapyr and imazamox) at 30g/ha when plants were at the 4-leaf stage. A second application of ODYSSEY™ (30g/h) was made when plants were in the rosette stage. Doubled haploid lines showing herbicide injury were noted. Observations recorded included: days to flowering, days to maturity, agronomic score at flowering and agronomic score at maturity. At physiological maturity, lines to be harvested were selected visually. A 20 g seed sample was harvested from each of the selected lines. The quality check rows of 46A72 were also harvested. The samples were analyzed in the lab and for oil percentage, protein percentage, and total glucosinolates using NIR (Near Infrared Spectroscopy). Final selection of lines was based on days to maturity, agronomic score at maturity, oil percentage, protein percentage and total glucosinolates. Several doubled haploid lines were selected including 97DHS-6259.

Generation:	Greenhouse Pure seed increase
Seed Planted:	97DHS-6259
Seed Harvested:	97DHS-6259
Method:	Each selected line including 97DHS-6259, was planted in the greenhouse (project SN-707) using remnant pure seed. All lines were sprayed with 60 g/ha ODYSSEY™ (2x rate) at the 4-leaf stage, in order to confirm imidazolinone resistance. All lines were inoculated with blackleg (<i>Phoma lingam</i>) spores, to induce disease development. Lines showing herbicide injury and/or susceptibility to blackleg were discarded. Selected lines, including 97SN-6259 were self-pollinated to produce 20g of seed, and were assigned new code numbers. 97SN-6259 was assigned the code, NS3801.

Generation: Field Evaluation (R200 tests)

Seed Planted: NS3801

Seed Harvested: NS3801

Method: The selected lines including NS3801, were evaluated in a two replicate yield trial (R221) planted at six locations in western Canada. Plot size was 9 square meters (6m x 1.5 m). The seeding rate was 5.5 kg/ha. Appropriate check varieties were included in the yield trial. The same entries were planted in a disease trail where blackleg inoculum was applied to ensure uniform disease infection. Observations recorded included: days to flowering, days to maturity, lodging score (1=poor, 9=good), yield (q/ha), and moisture percentage. At harvest, a 15 gram seed sample was collected from each plot, and was analyzed to determine oil percentage, protein percentage, total glucosinolates, and fatty acid composition.

Table 1 illustrates the performance of *Brassica napus* variety NS3801 in comparison to WCC/RRC check varieties.

VARIETY	Yield** (Qu/Ha)	Yield (% Chk)	Maturity (Days)	Oil (%)	Protein (%)	Glucs (uM/g)	Blackleg (1-9)**
NS3801	29.60	93.36	102.40	48.29	46.30	13.89	8.44
Defender	29.67	93.80	105.70	48.16	48.14	13.63	6.57
Excel	33.93	107.27	109.50	49.70	47.67	18.08	6.04
Legacy	31.34	99.08	108.40	49.64	48.75	10.90	5.57
Mean of Napus Chks #	31.65	100.05	107.87	49.17	48.19	14.20	6.06
Difference	-2.05	-6.69	-5.47	-0.88	-1.89	-0.31	2.38

* Data from Pioneer Hi-Bred Trials in the Short Season Zone of Western Canada

** Trait Definitions: Yield = seed yield in quintals (decitonnes) per hectare and as percentage of Checks Mean; Maturity = days from Planting to physiological maturity; Oil & Protein as percentage of total seed weight at 8.5% moisture; Glucs = aliphatic glucosinolates in seed at 8.5% moisture, expressed in micromoles per gram

WCC/RRC Check Varieties for B. napus & B. rapa. For registration of early B. napus varieties, yield and composition are compared to B. rapa, and maturity is compared to B. napus, where Early Napus = -4 days or more vs. B. napus checks

DEPOSITS

This invention is not to be construed as limited to the particular embodiments disclosed, since these are regarded as illustrative rather than restrictive. Moreover, variations and changes may be made by those skilled in the art without departing
5 from the spirit of this invention.

The seeds of the subject invention were deposited in the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, USA:

Seed	Accession Number	Deposit Date
<i>Brassica napus</i> NS3801	PTA-2470	September 14, 2000

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
219